

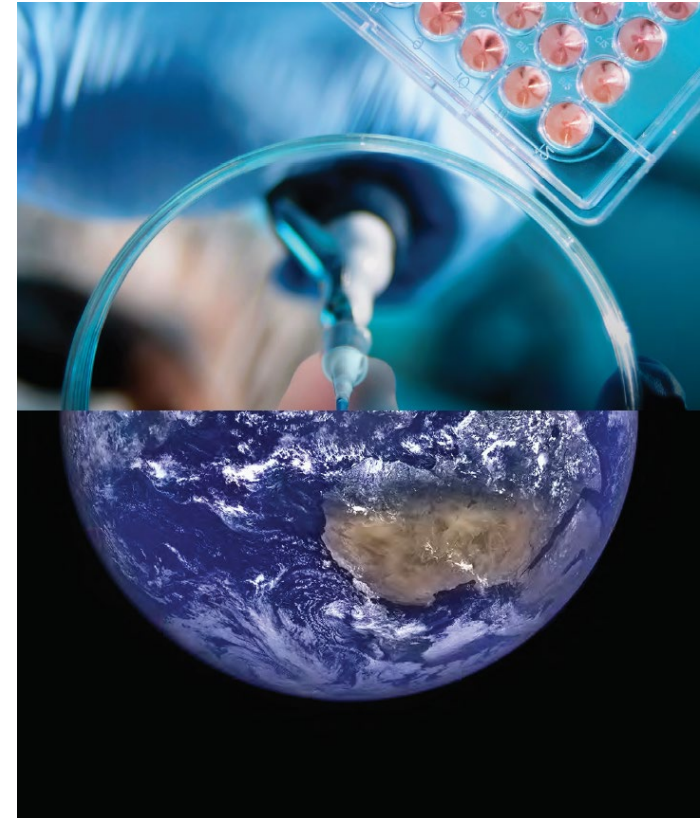
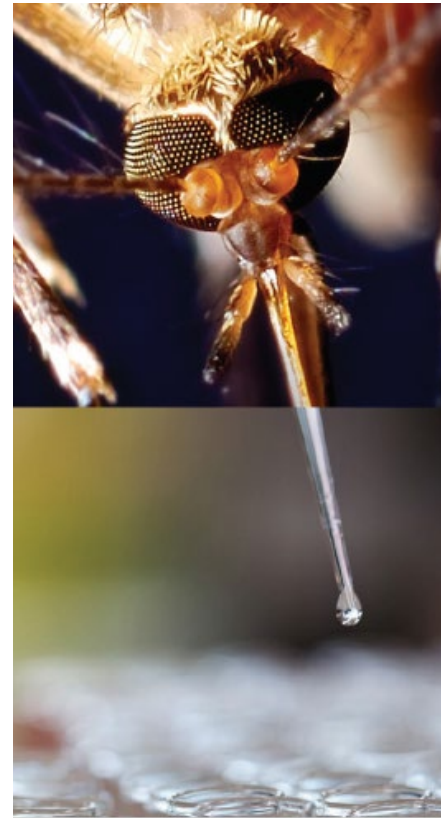
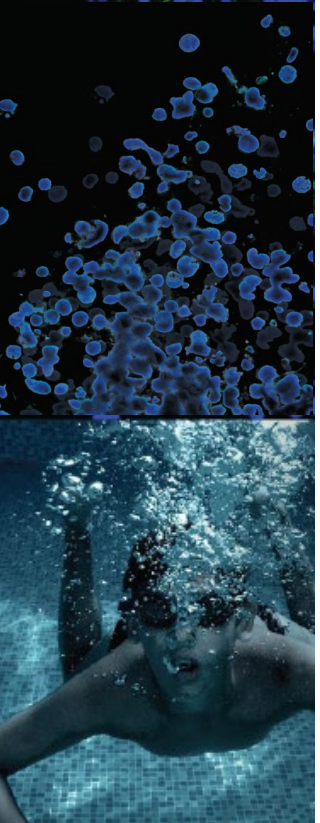


ATCC® NGS Standards and Their Applications

How Standards Help Establish Reliable Workflows for Microbiome Analysis

Leka Papazisi, DVM, PhD
Principal Scientist, ATCC

Credible Leads to InCredible™



About ATCC®

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
 - 5,000 cell lines
 - 80,000 microorganisms
 - Genomic & synthetic nucleic acids
 - Media/reagents
- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees



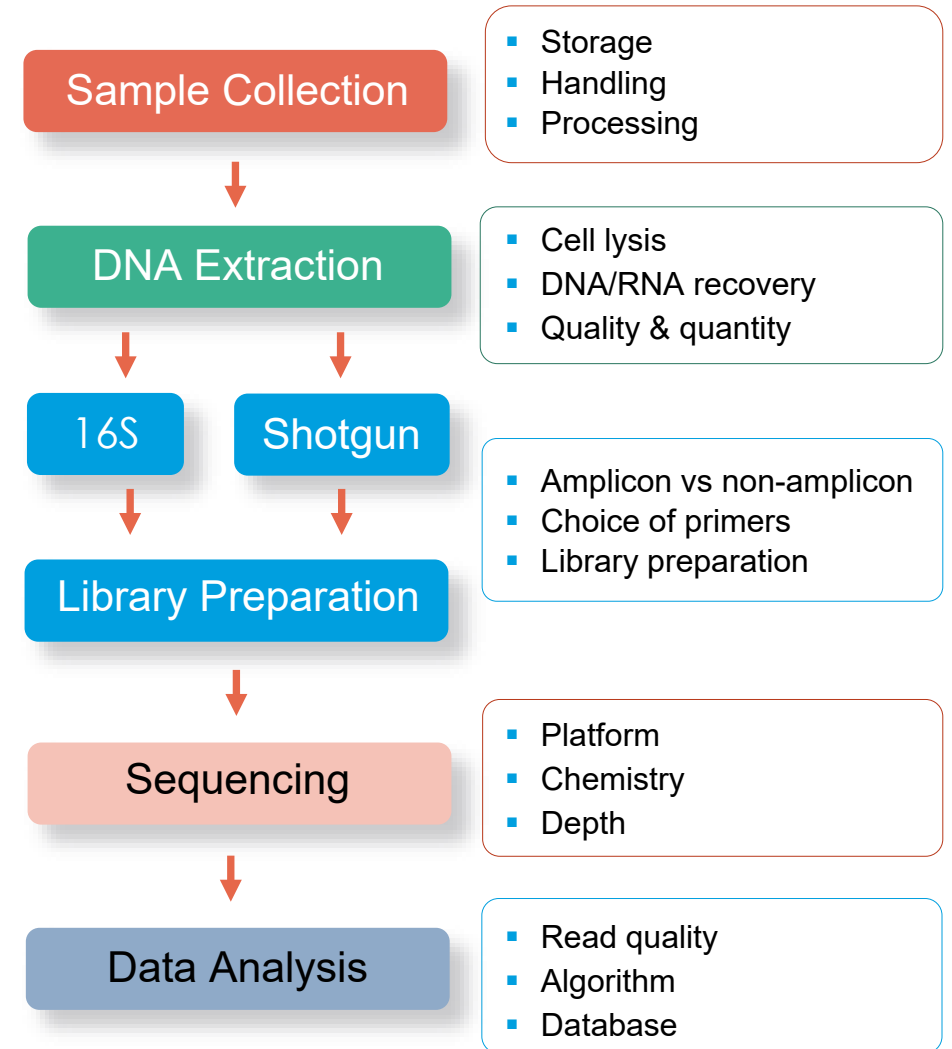
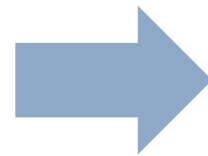
Microbiome Research

The microbiome field is rapidly moving toward translational research pertinent to human health and disease, therapeutics, and personalized medicine


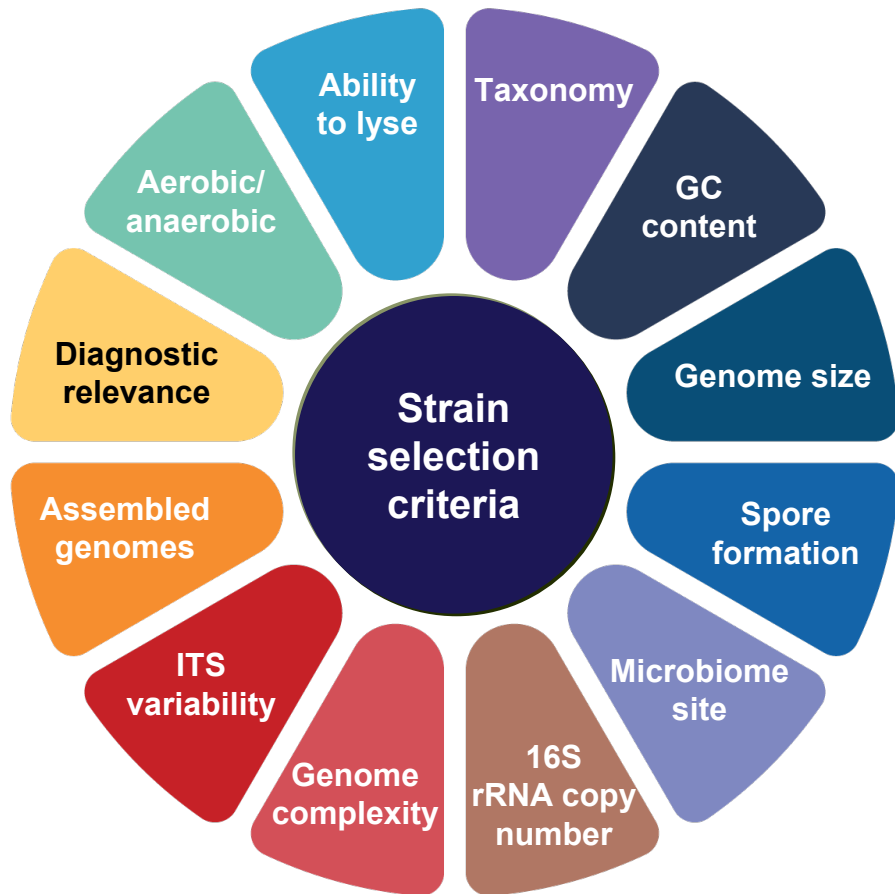
Challenges in Microbiome Research and Applications

sequencing viability coverage
extraction technology
amplification
depth bioinformatics

bias

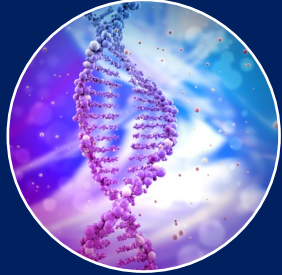


Development of ATCC NGS Standards



Whole Cell Standards

- Authenticated ATCC cultures
- Growth and image cytometry cell counting
- Mixed in even proportion-based cell numbers cells
- Storage at 4°C



Genomic DNA Standards

- Authenticated ATCC nucleic acids
- Fluorescent dye-based quantification
- Mixed in even proportions-based genome copy number
- Storage at -20°C

← Assay development, optimization, verification, and quality control →

ATCC® NGS Standards Portfolio



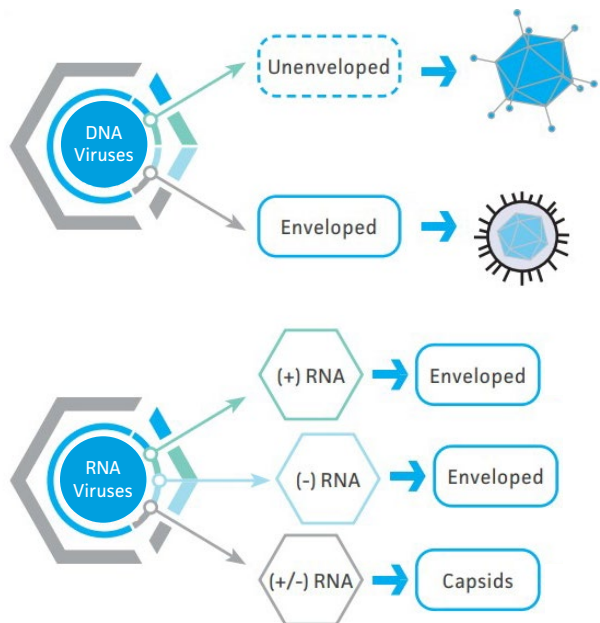
Preparation	ATCC® Catalog No.	Number of Organisms	Composition	Complexity	Utility
Genomic DNA	MSA-1000™	10	Even	Medium	Standards for assay development and optimization
	MSA-1001™	10	Staggered	Medium	
	MSA-1002™	20	Even	High	
	MSA-1003™	20	Staggered	High	
Whole cell	MSA-2003™	10	Even	Medium	
	MSA-2002™	20	Even	High	
Genomic DNA	MSA-4000™	11	Staggered	Medium	NGS-based pathogen detection
Genomic DNA	MSA-3000™	6	Even	Low	Environmental studies
	MSA-3001™	10	Even	Medium	
	MSA-3002™	10	Staggered	Medium	

ATCC® Site-specific NGS Standards



Standard	Preparation	ATCC® Catalog No.	Number of Organisms	Importance
Oral	Whole cell	MSA-2004™	6	<ul style="list-style-type: none"> Mock microbial communities representing the oral, skin, gut, and vaginal microbiomes Comprises normal and atypical flora Anaerobic and aerobic microbial strains A combination of Gram-positive and Gram-negative bacterial cultures Even composition
	Genomic DNA	MSA-1004™		
Skin	Whole cell	MSA-2005™	6	
	Genomic DNA	MSA-1005™		
Gut	Whole cell	MSA-2006™	12	
	Genomic DNA	MSA-1006™		
Vaginal	Whole cell	MSA-2007™	6	
	Genomic DNA	MSA-1007™		

ATCC® Virome Standards



Composition of Virome Standards

Human herpesvirus 5 strain AD169 (ATCC® VR-538™)

Human mastadenovirus strain F (ATCC® VR-931™)

Influenza B virus strain B/Florida/4/2006 (ATCC® VR-1804™)

Zika virus strain MR 766 (ATCC® VR-1838™)

Reovirus 3 strain Dearing (ATCC® VR-824™)

Human respiratory syncytial virus strain A2 (ATCC® VR-1540™)

Standard	Preparation	ATCC® Catalog No.	Number of Organisms	Specification (ddPCR™)	Applications
Virome	Virus Mix	MSA-2008™	6	2×10^3 genome copies/ μL per virus	Standards for virome assay development, optimization, verification, and validation; evaluating reproducibility; and use as a daily run quality control
	Nucleic Acid Mix	MSA-1008™	6	2×10^4 genome copies/ μL per virus	

ATCC® Spike-in and Mycobiome Standards



Standard	Preparation	ATCC® Catalog No.	Number of Organisms	Application
Spike-in	Whole cell	MSA-2014™	3	<ul style="list-style-type: none"> Microbiome measurements and data normalization 16S rRNA and shotgun assay verification, validation, and quality control
	Genomic	MSA-1014™		



Standard	Preparation	ATCC® Catalog No.	Number of Organisms	Application
Mycobiome	Whole cell	MSA-2010™	10	<ul style="list-style-type: none"> Fungal mock community standards for assay development, optimization, verification, and validation; evaluating reproducibility; and use as a daily run quality control
	Genomic	MSA-1010™		

ATCC® Genome Portal

A cloud-based platform that enables users to easily browse authenticated and traceable reference genomes and metadata.



Download whole-genome sequences and annotations of ATCC materials



Search for nucleotide sequences or genes within genomes



View genome assembly metadata and quality metrics

genomes.atcc.org

Yarmosh DA, et al. Comparative Analysis and Data Provenance for 1,113 Bacterial Genome Assemblies. *MSphere* 7(3): e00077-22, 2022

3,238 Authenticated Microbial Reference Genomes

2,778 bacteria
250 viruses
206 fungi
4 protists

New genomes released every month!

REST-API for bioinformatics applications available

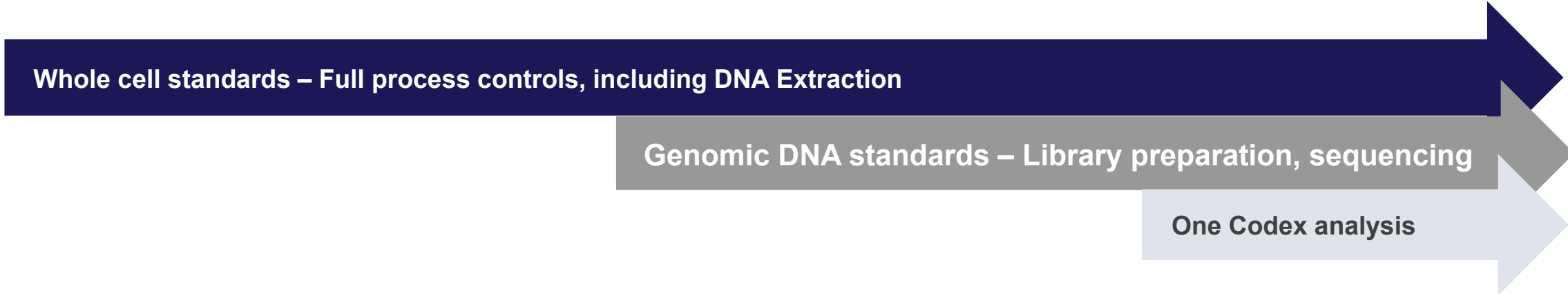
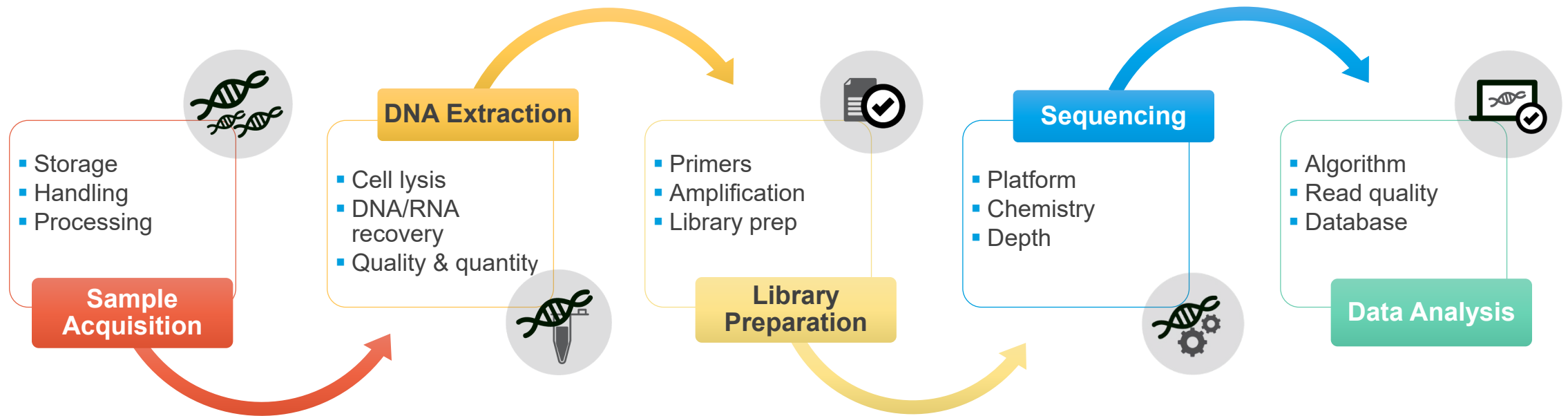
Free for non-commercial research use (RUO) purposes. Commercial use licenses available. Registration required.



Utility and Application of NGS Standards

- Evaluating DNA extraction methods and kits
- Evaluating 16S rRNA and WGS library kits
- Evaluating NGS platforms

Microbiome Workflow, Biases, and Standardization



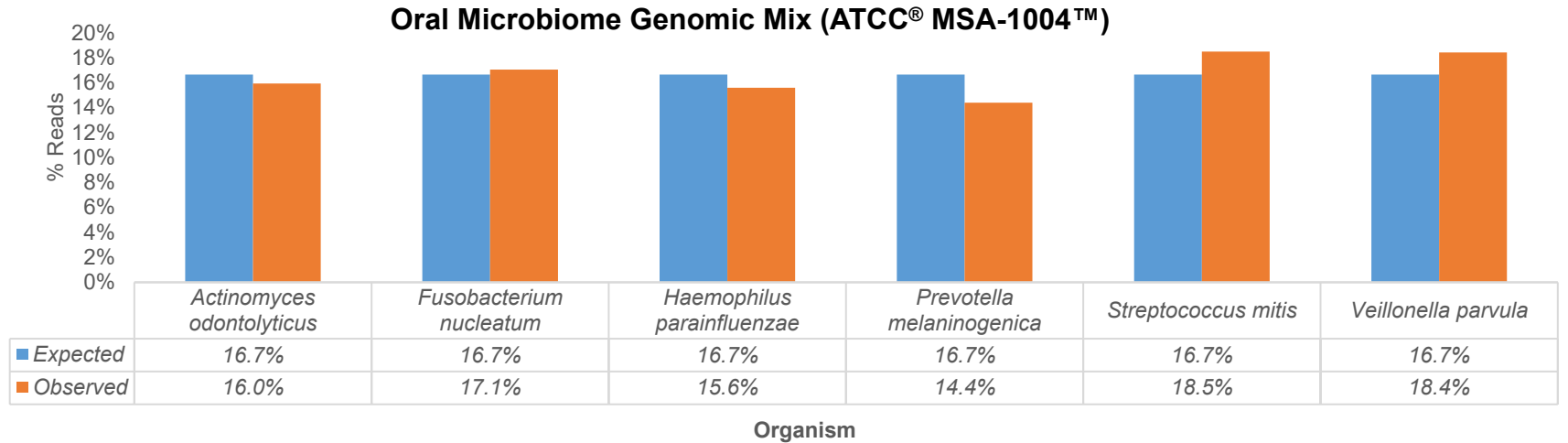


Evaluating DNA Extraction Methods and Kits

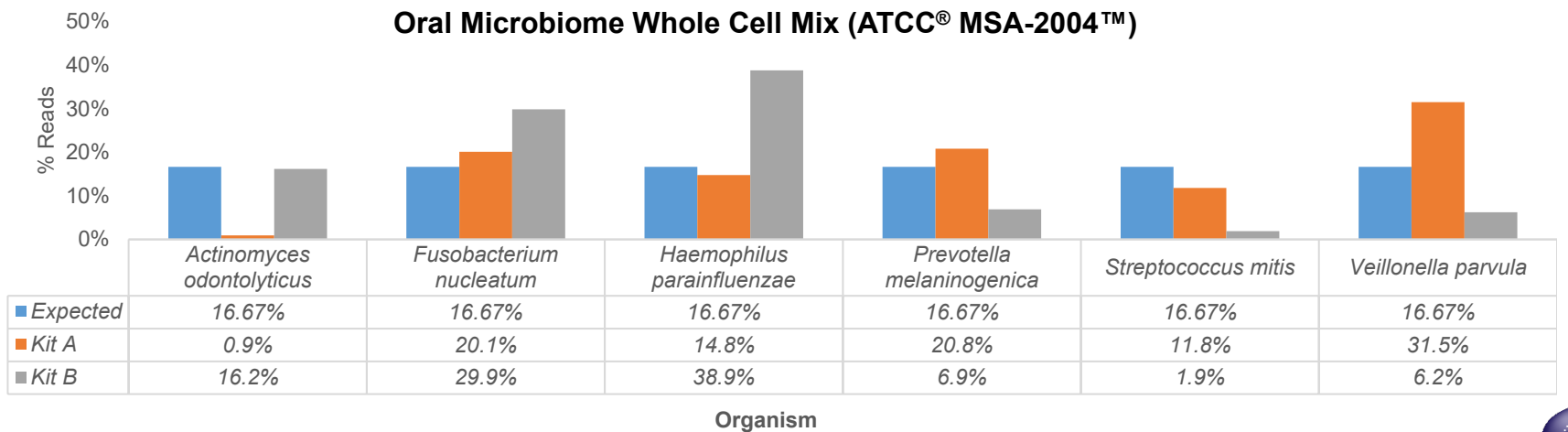
Genomic Versus Whole Cell Standards

DNA extraction methods are not perfect

Shotgun metagenomic analysis of the Oral Microbiome Genomic Mix



DNA extraction from the Oral Microbiome Whole Cell Mix with two different kits followed by shotgun metagenomic analysis



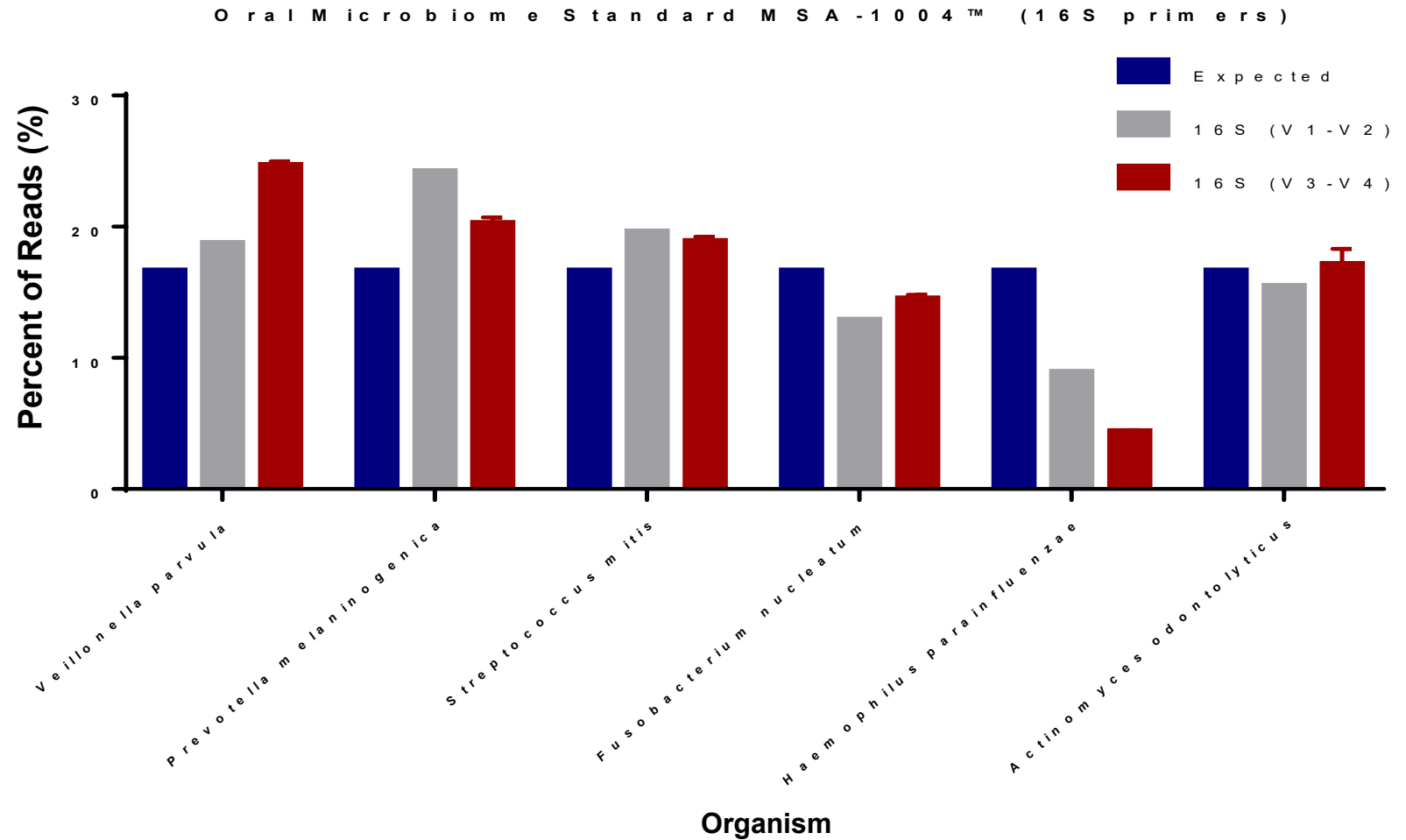


Evaluating 16S rRNA and WGS Library Kits

16S Amplicon-based Analysis: Primer Selection

Compare different primer sets, optimize amplification steps, and validate 16S analysis methods

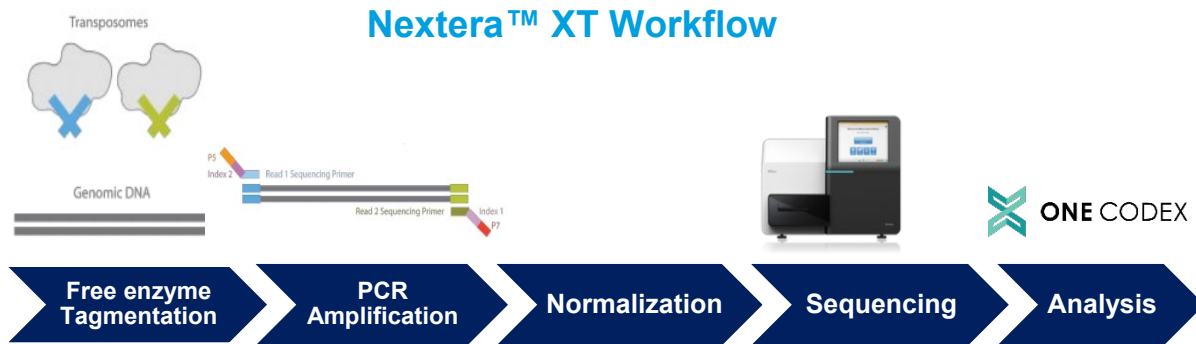
16S rRNA analysis of the Oral Genomic DNA Standard via two primer sets



Comparing Library Preparation Kits

Nextera Flex enables uniform coverage of genomes of low GC content

Oral Microbiome Genomic DNA (ATCC® MSA-1004™)



Sample Composition

Name	Estimated Abundance
Actinomyces odontolyticus	46.07%
Prevotella melaninogenica	16.09%
Streptococcus mitis	16.04%
Veillonella parvula	12.65%
Haemophilus parainfluenzae	7.80%
Fusobacterium nucleatum	1.34%



Sample Composition

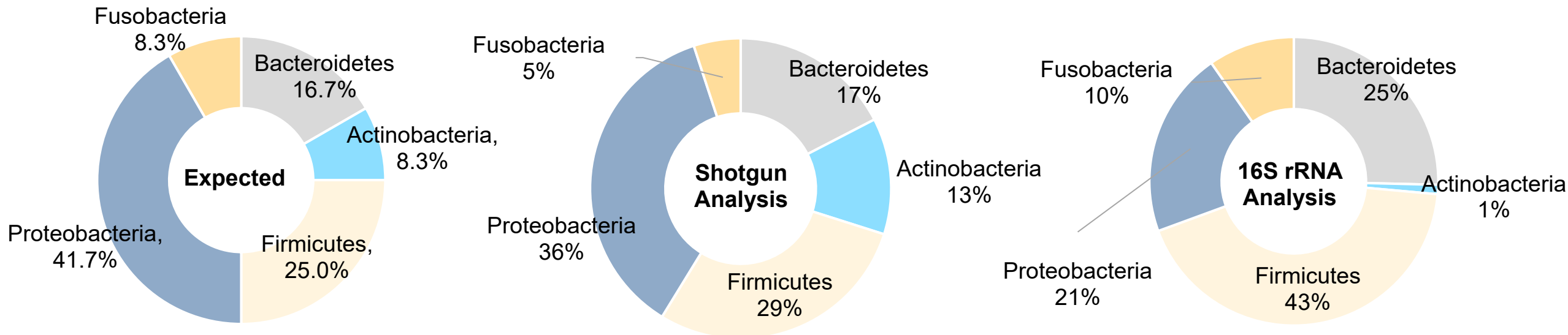
Name	Estimated Abundance
Streptococcus mitis	18.83%
Veillonella parvula	18.42%
Fusobacterium nucleatum	17.13%
Haemophilus parainfluenzae	15.68%
Actinomyces odontolyticus	15.54%
Prevotella melaninogenica	14.40%



Gut Whole Cell Standard

Profiling of gut microbiome standard at the phylum, genus, and species level

The Gut Whole Cell Microbiome Standard (ATCC[®] MSA-2006[™]) can be used as a full process control for shotgun and 16S rRNA assays



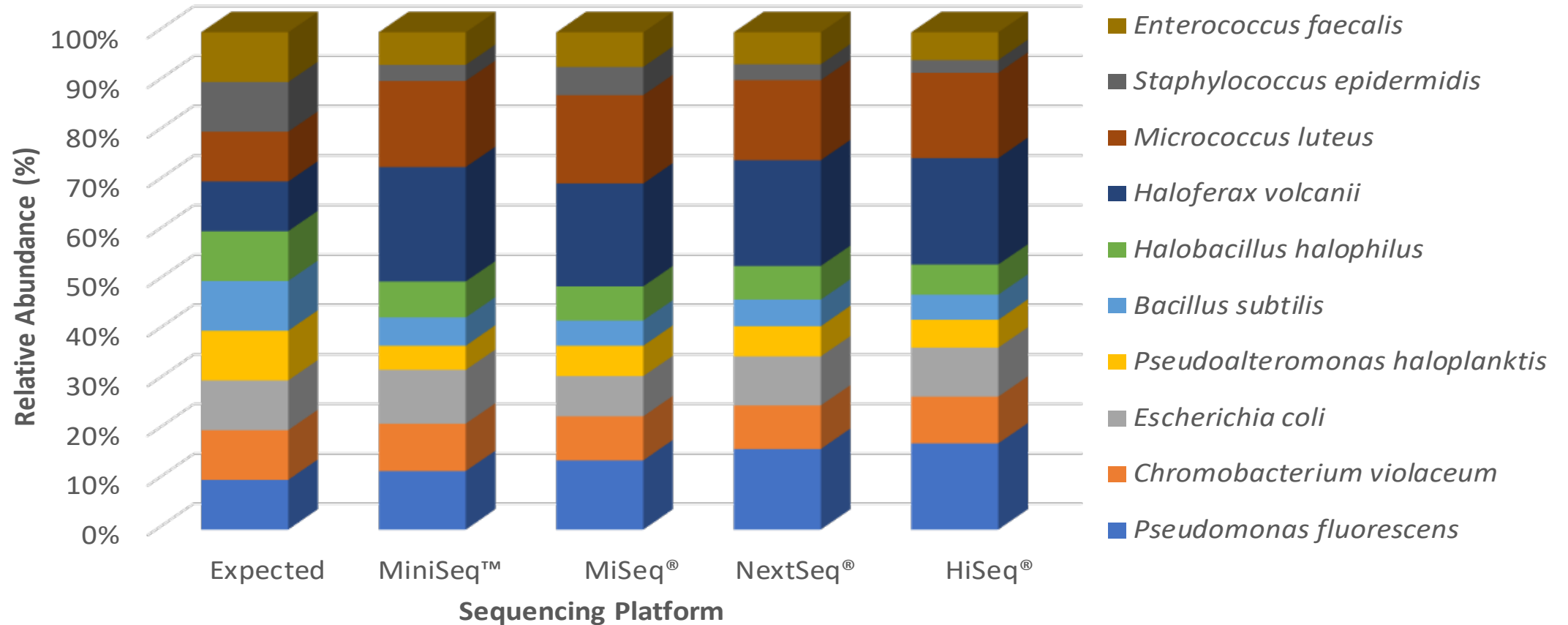


Evaluating NGS Platforms

Short-read Sequencing Platform: Illumina®

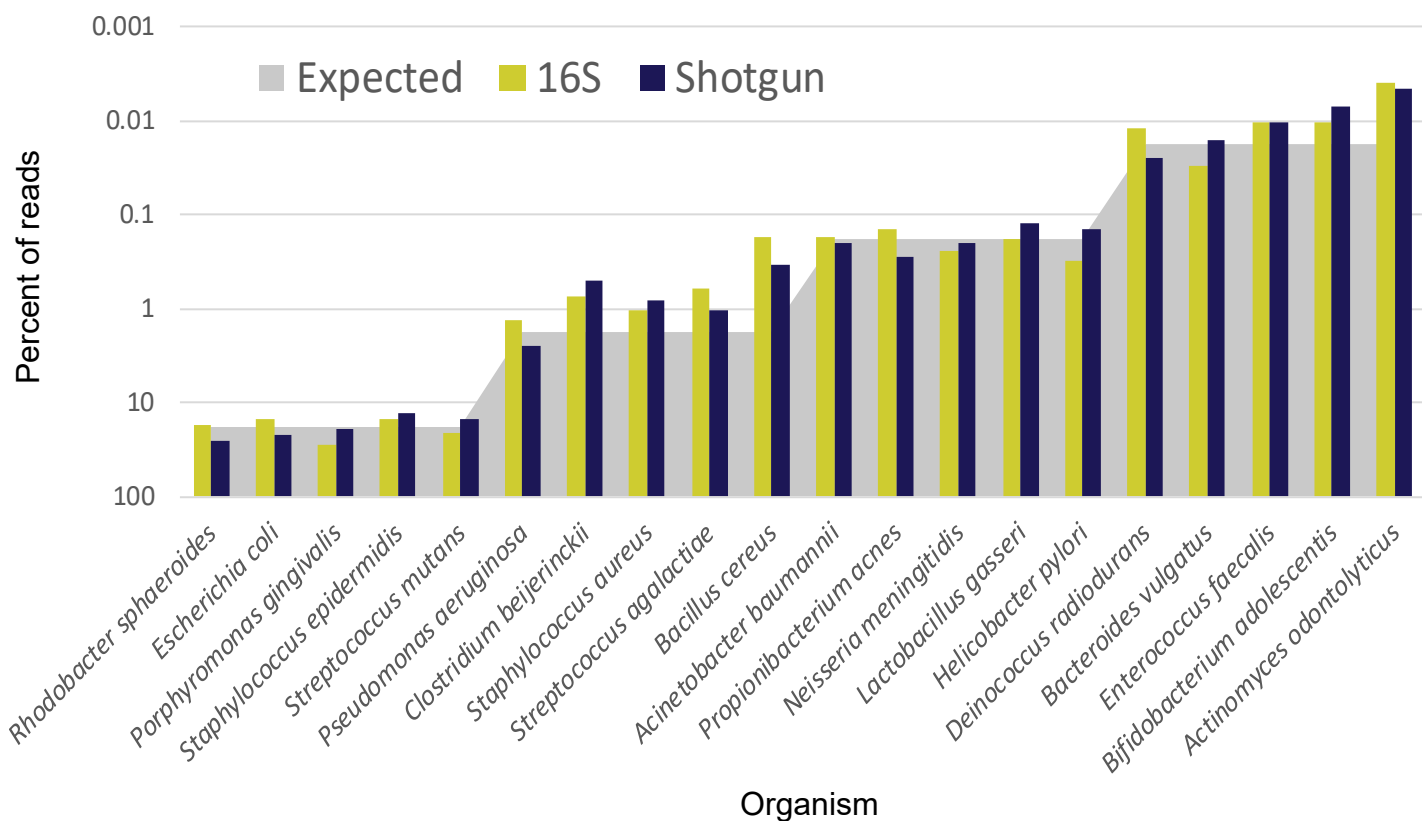
Assay reproducibility through different Illumina sequencing platforms

Shotgun Metagenomic Data (ATCC® MSA-3001™)



Long-read Sequencing Platform: PACBIO®

16S rRNA (full-length) and shotgun data on the PacBio Sequel Platform using ATCC® MSA-1003™



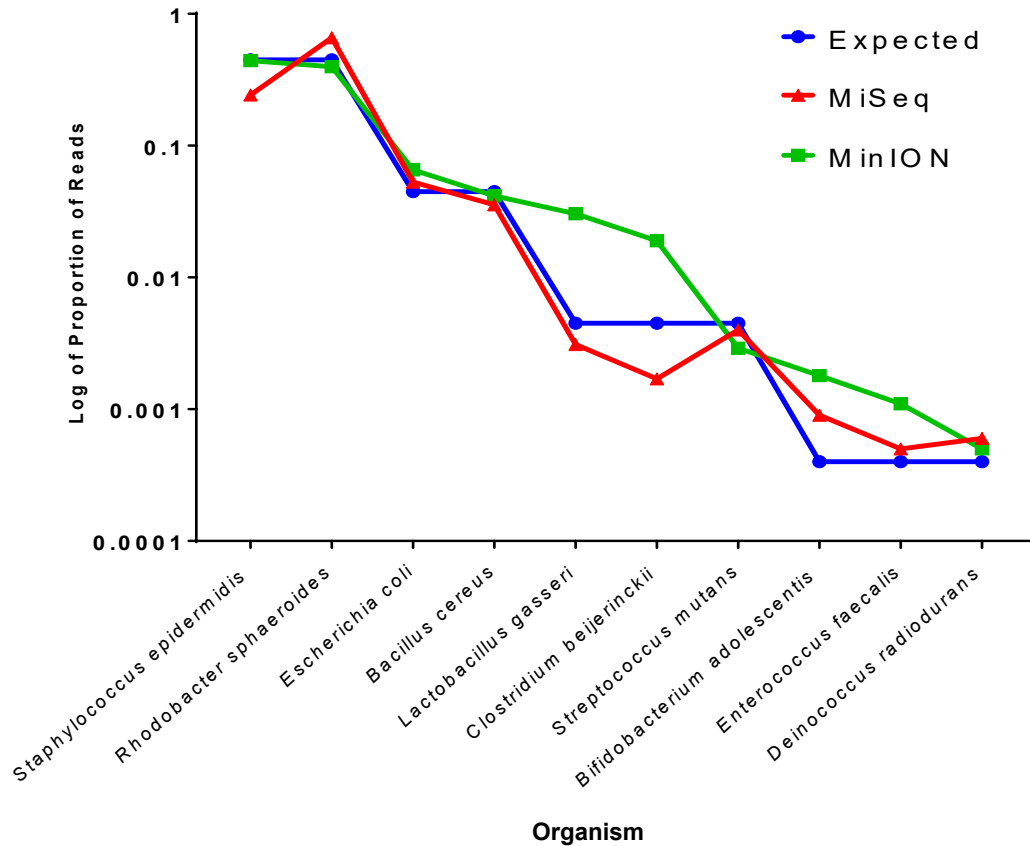
ATCC quality control score (One Codex)

One Codex Analysis	16S rRNA run 1	16S rRNA run 2	Shotgun run 1	Shotgun run 2
True positives	100%	100%	100%	100%
Relative abundance	95%	95%	97%	97%
False positives	0%	0%	12%	16%
Overall score	98%	98%	95%	95%

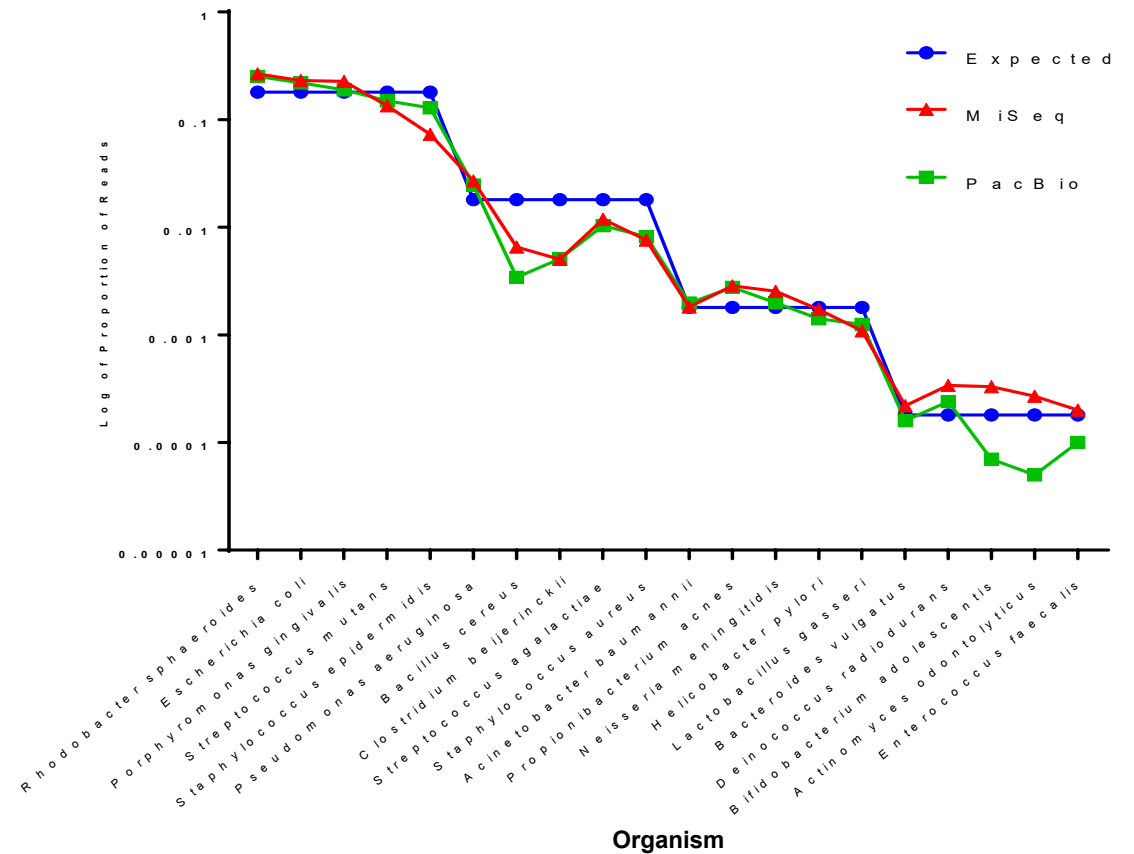
Shotgun Metagenomic Analysis: Short vs Long Reads

ATCC® NGS Standards are technology agnostic

MSA-1001™ (Staggered 10 Strains)



MSA-1003™ (Staggered 20 Strains)





Fungal Mock Community Standards for Mycobiome Studies

Mycobiome Research: An Emerging Need for Reference Material

frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 13 February 2015
doi: 10.3389/fmicb.2015.00089



The lung mycobiome: an emerging field of the human respiratory microbiome

Linh D. N. Nguyen¹, Eric Viscogliosi¹ and Laurence Delhaes^{1,2*}

Cui et al. *Genome Medicine* 2013, 5:63
<http://genomemedicine.com/content/5/7/63>



REVIEW

The human mycobiome in health and disease

Lijia Cui¹, Alison Morris² and Elodie Ghedin^{1,3*}

Review Article

The mycobiome of the human urinary tract: potential roles for fungi in urology

A. Lenore Ackerman¹, David M. Underhill²

Cell Article

Temporal Stability of the Human Skin Microbiome

Cell Host & Microbe Previews

Authors
Julia Oh, Allyson L. Byrd, Morgan Park, NISC Comparative Sequencing Program, Heidi H. Kong, Julia A. Segre



International Journal of
Molecular Sciences

Review

The Fungal Mycobiome and Its Interaction with Gut Bacteria in the Host

Qi Hui Sam¹, Matthew Wook Chang^{2,3} and Louis Yi Ann Chai^{1,4,*}



Through the Scope Darkly: The Gut Mycobiome Comes into Focus

Nydiaris Hernández-Santos¹ and Bruce S. Klein^{1,2,3,*}

Mycobiome Composition: Fungal Mock Community

Fungi strains selection attributes and clinical relevance

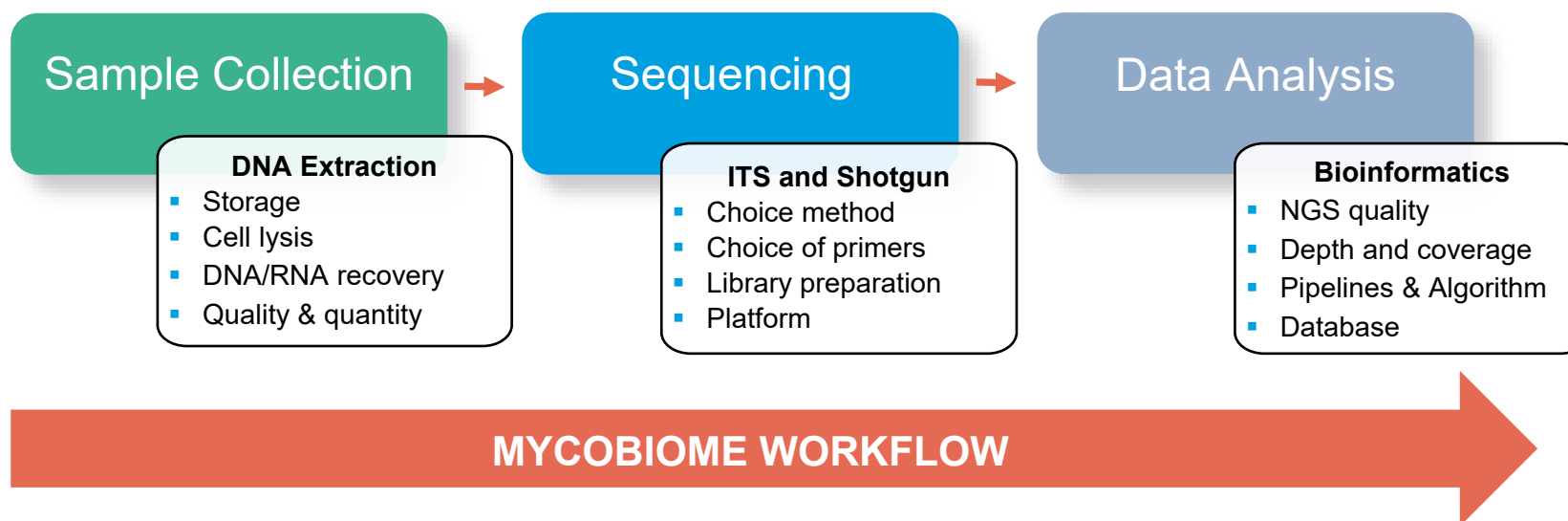
ATCC® No.	Species Name	Genome Size (Mb)	Relevancy
MYA-4609™	<i>Aspergillus fumigatus</i>	28.8	Opportunistic, airborne pathogen that is responsible for fungal infections in immunocompromised patients.
10231™	<i>Candida albicans</i>	17.1	Commensal fungus of the oral cavity that can form biofilms on denture surfaces, leading to mucosal infections.
2001™	<i>Candida glabrata</i>	12.3	Commensal fungus of the oral cavity and human gut that can acquire resistance to azole antifungals, leading to infection.
208821™	<i>Cryptococcus neoformans var. grubii</i>	18.9	Responsible for cryptococcal meningitis in immunosuppressed patients.
MYA-4612™	<i>Malassezia globosa</i>	9.0	Part of the normal skin flora but can be responsible for skin diseases such as dandruff, dermatitis, and folliculitis.
201390™	<i>Saccharomyces cerevisiae</i>	12.2	Bakers' and brewers' yeast originating in food. Emerging pathogen in immunocompromised patients.
9533™	<i>Trichophyton interdigitale</i>	21.9	Can infect skin and nails to cause chronic infections such as athlete's foot and ringworm.
204094™	<i>Cutaneotrichosporon dermatis</i> (<i>Trichosporon dermatis</i>)	23.3	Emerging opportunistic agent of invasive fungal infections, particularly in severely immunocompromised patients.
10106™	<i>Penicillium chrysogenum</i>	32.5	Spore-former, less prevalent, but can be responsible for intestinal infection in immunosuppressed patients.
36031™	<i>Fusarium keratoplasticum</i> (<i>F. solani</i> complex)	48.6	Filamentous, opportunistic pathogen that causes fungal keratitis.

ATCC® Mycobiome NGS Standards

Product description, research use, and applications



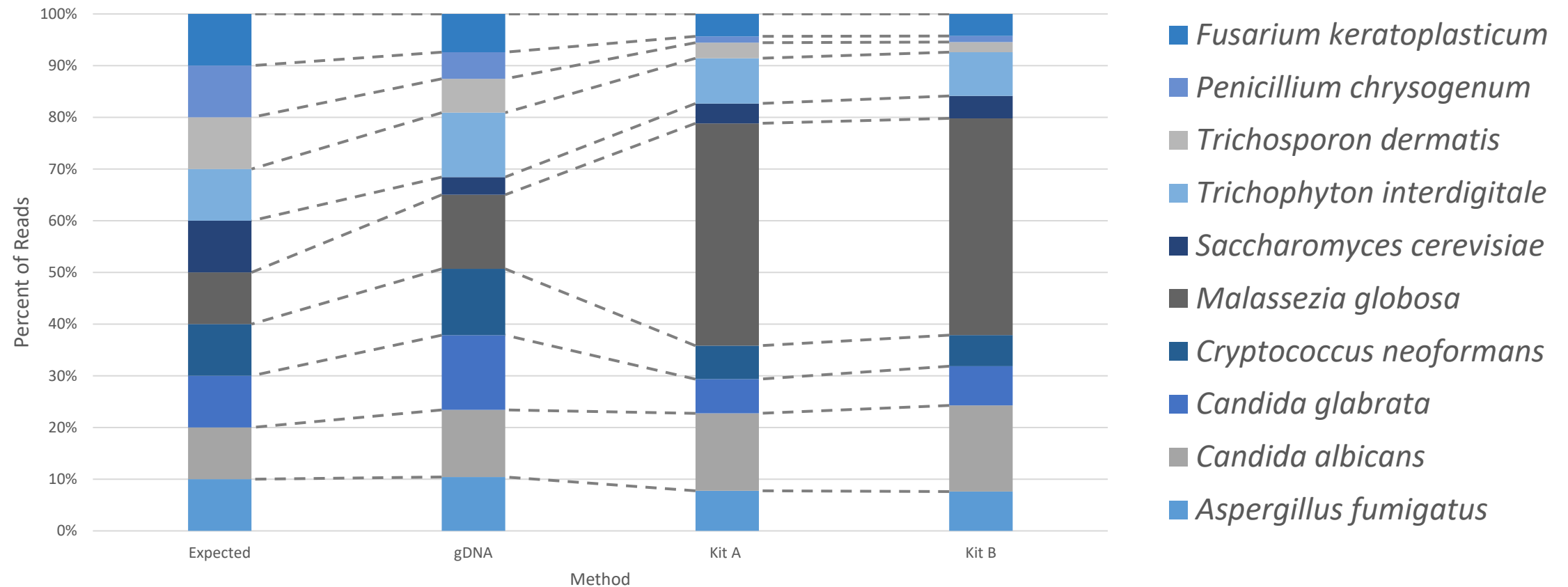
Standard	ATCC® Catalog No.	Preparation	Description
Mycobiome	MSA-2010™	Whole cell	Even mixture of whole cells comprising 10 fungal strains (2 x 10 ⁶ cells of each organism)
	MSA-1010™	Genomic DNA	Even mixture of genomic DNA comprising 10 fungal strains (2 x 10 ⁶ genome copies of each organism)



Mycobiome Standards: Evaluation of DNA Extraction kits

Challenges with the development of whole cell standards and fungal DNA extraction methods

Shotgun Metagenomic Analysis of Mycobiome Standards (ATCC[®] MSA-2010[™] and MSA-1010[™])



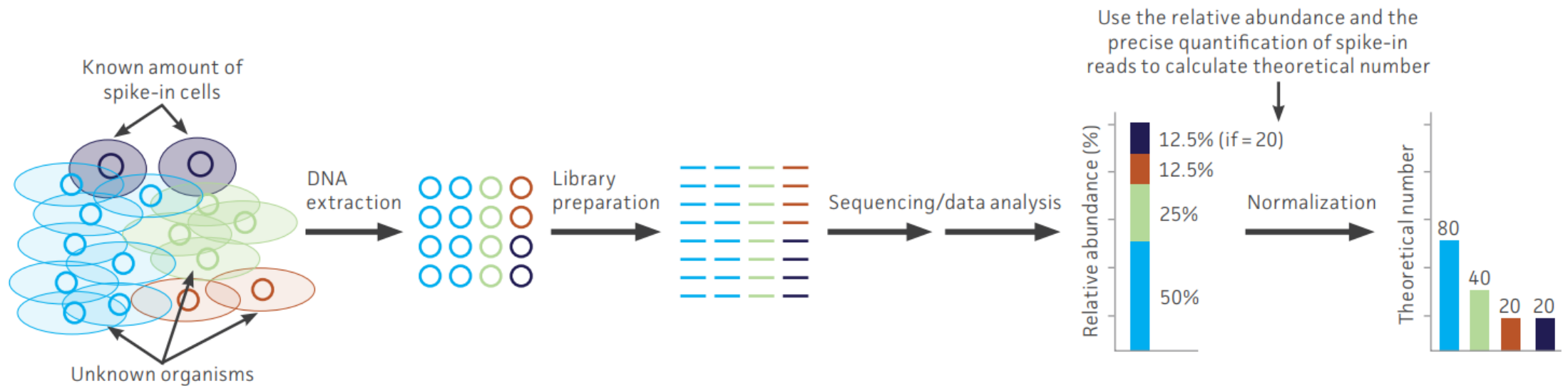
Conclusions about the ATCC Mycobiome Standards

- Whole cell standards can help identify biases introduced during DNA extraction and can be used as full-process controls
- Genomic DNA standards can be used for comparing various library preparation methods and sequencing platforms
- The data analysis for mycobiome profiling is challenging due to the lack of complete fungal reference genomes and the limited availability of analyses pipelines



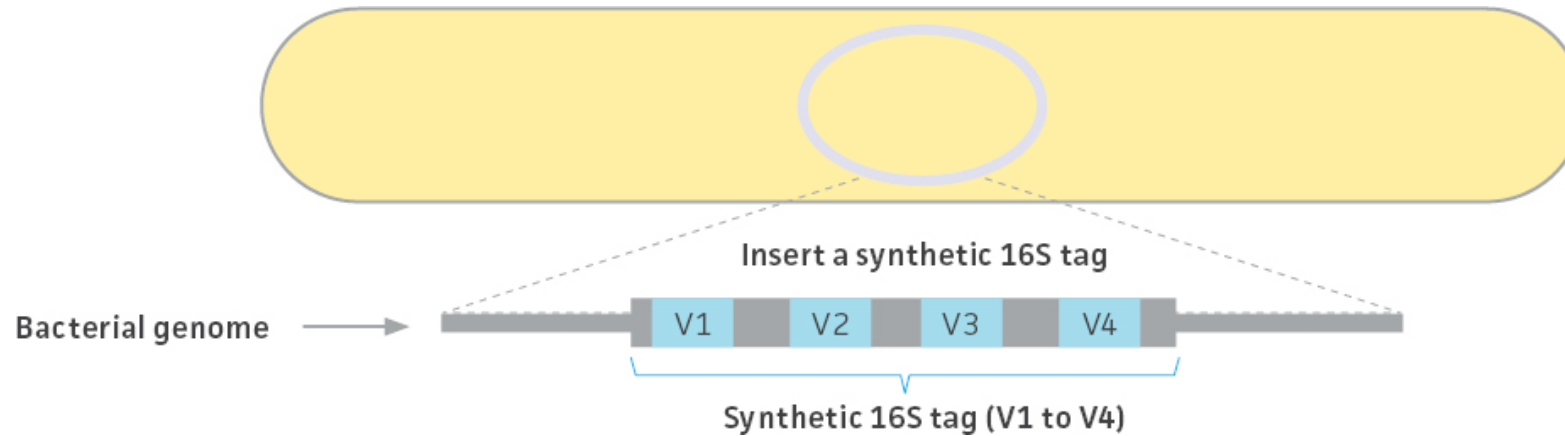
Spike-in Internal Controls: Synthetic 16S Tagged Strains

The Output of a Metagenomic Data is a Relative Abundance

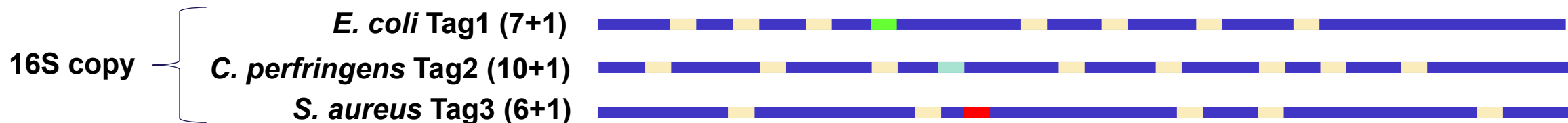


Relative abundance does not reflect the quantity of the microbial community and the inter-sample differences among taxa.

Engineering Synthetic 16S Tag Into Bacterial Genome



Bacterial Strains	Gram Stain	Genome G/C (%)	Insertion loci	BSL	
<i>Escherichia coli</i> Tag1	Negative	50.8	Beta galactosidase	1	T1
<i>Clostridium perfringens</i> Tag2	Positive	29.0	Theta-toxin	2	T2
<i>Staphylococcus aureus</i> Tag3	Positive	32.8	O-antigen polymerase	2	T3



Spike-in Standards (3 Strain Tagged Mix)

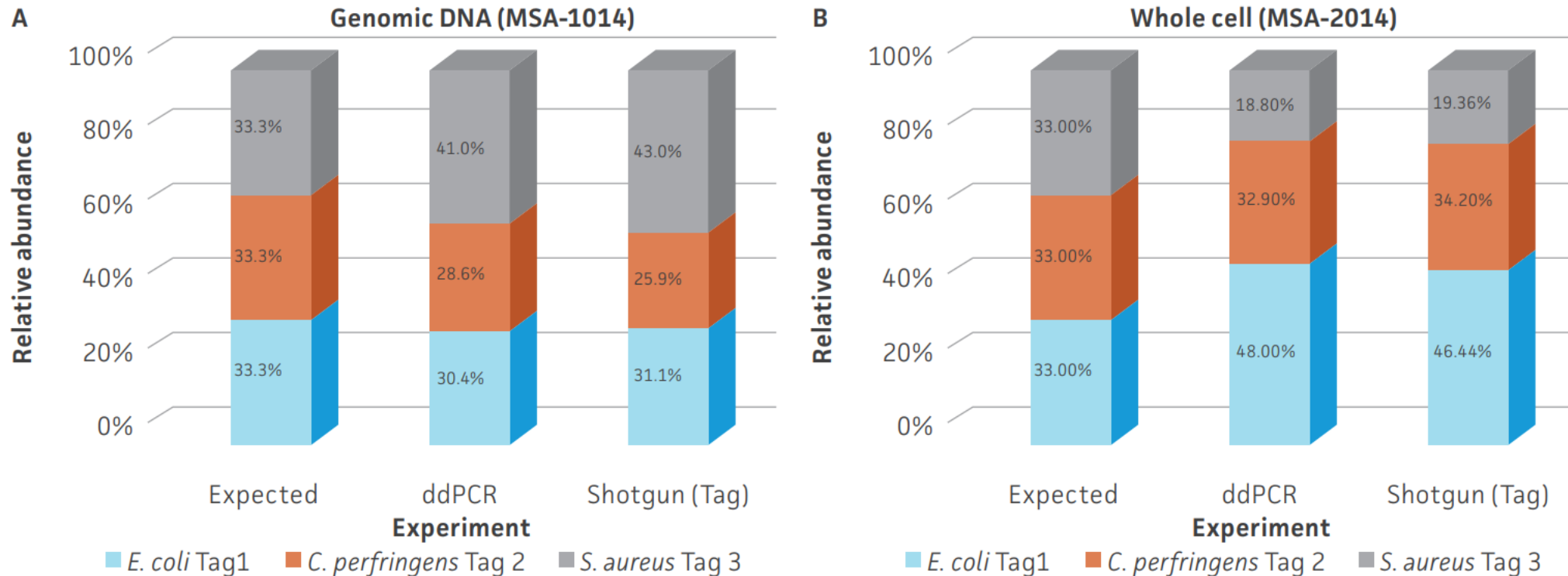


ATCC® Catalog No.	Preparation	Specification	Importance
MSA-1014™	Genomic DNA	6 x 10 ⁷ genomes copies/vial ± 1 log	<ul style="list-style-type: none"> Microbiome measurements and data normalization 16S rRNA and shotgun assay verification, validation, and quality control
MSA-2014™	Whole cells	6 x 10 ⁷ cells/vial ± 1 log	

Spike-in Composition

Species	Gram Stain	Genome size (Mb)	Tag size (bp)	G/C Content (%)	16S Copies	Tag copies	Cells per vial
<i>Escherichia coli</i> Tag1	Negative	4.59	829	50.8	7	1	2 x 10 ⁷
<i>Clostridium perfringens</i> Tag2	Positive	3.25	799	29.0	10	1	2 x 10 ⁷
<i>Staphylococcus aureus</i> Tag3	Positive	2.70	833	32.8	6	1	2 x 10 ⁷

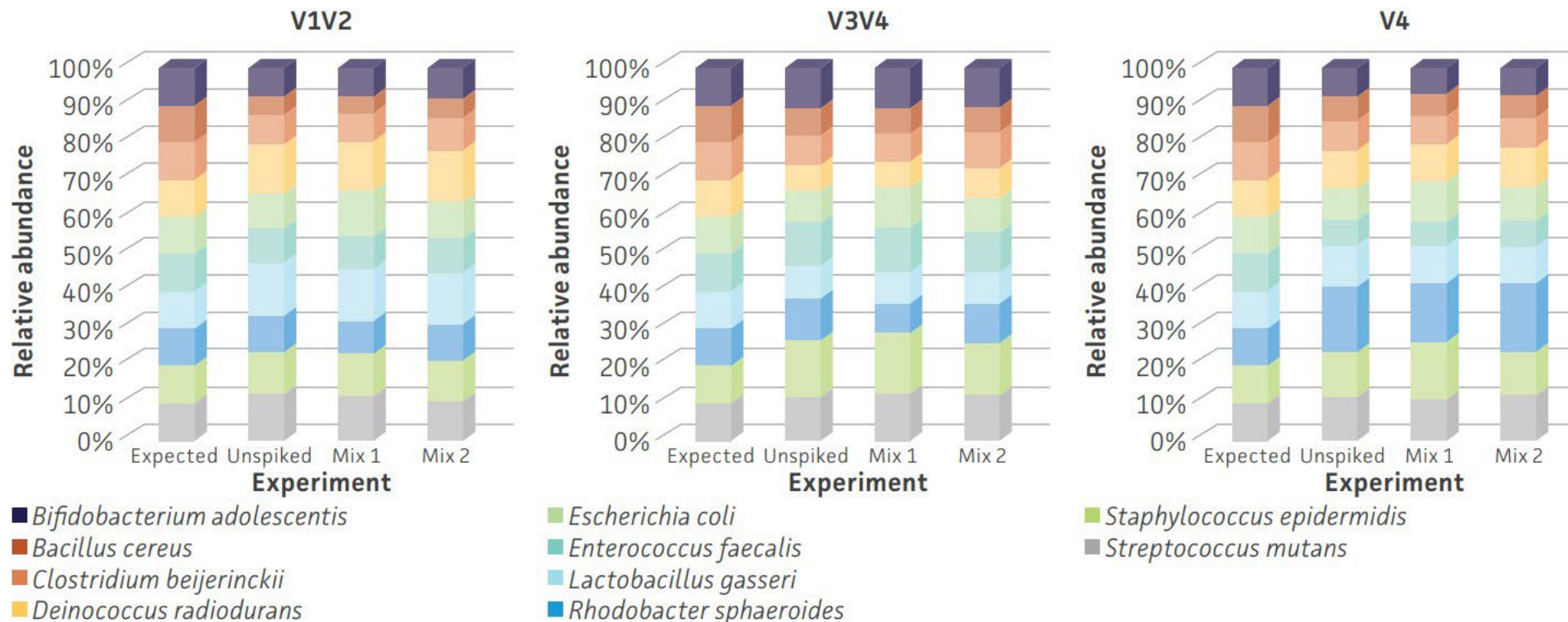
Development of Spike-in Standards and Quality Control



Relative abundance of the genomic DNA and whole cell spike-in standards

Relative Abundance of a Mock Community with Spike-in

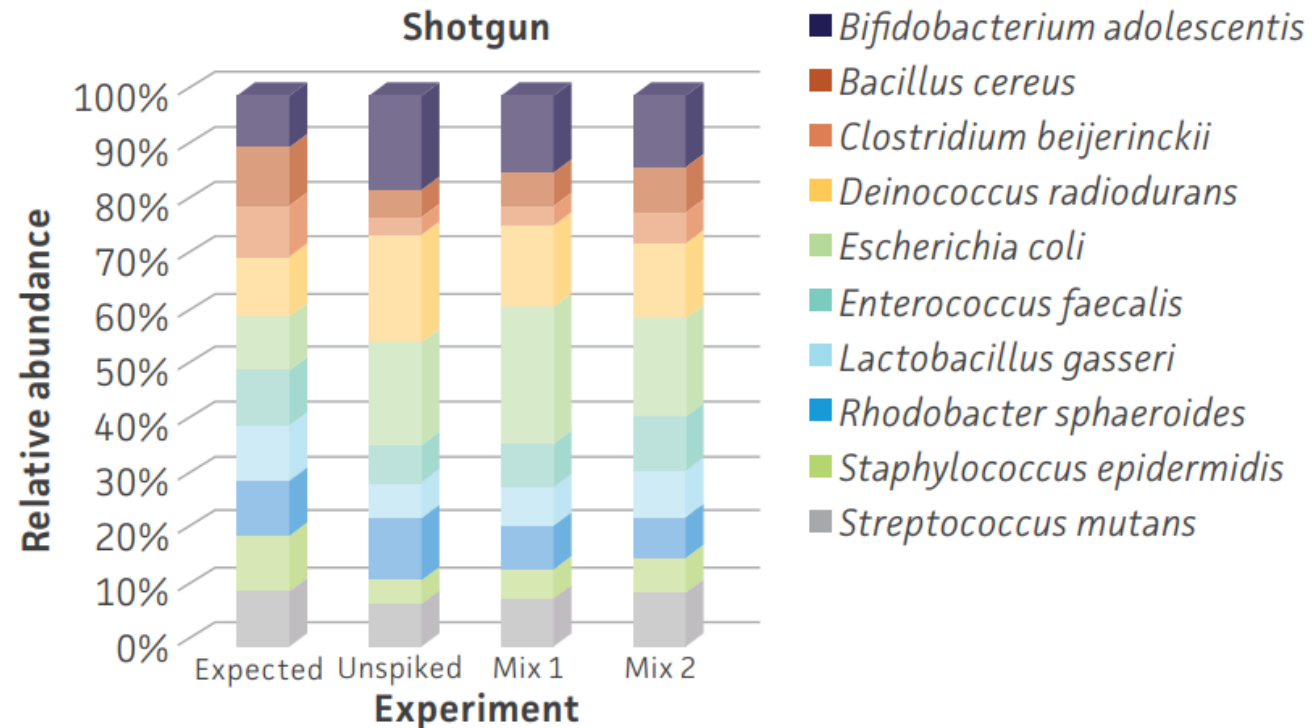
The spike-in doesn't have obvious impact on 16S relative abundance



ATCC[®] MSA-1014[™] was mixed with MSA-1000[™] at ~ 1:10 and ~1:100

Relative Abundance of a Mock Community with Spike-in

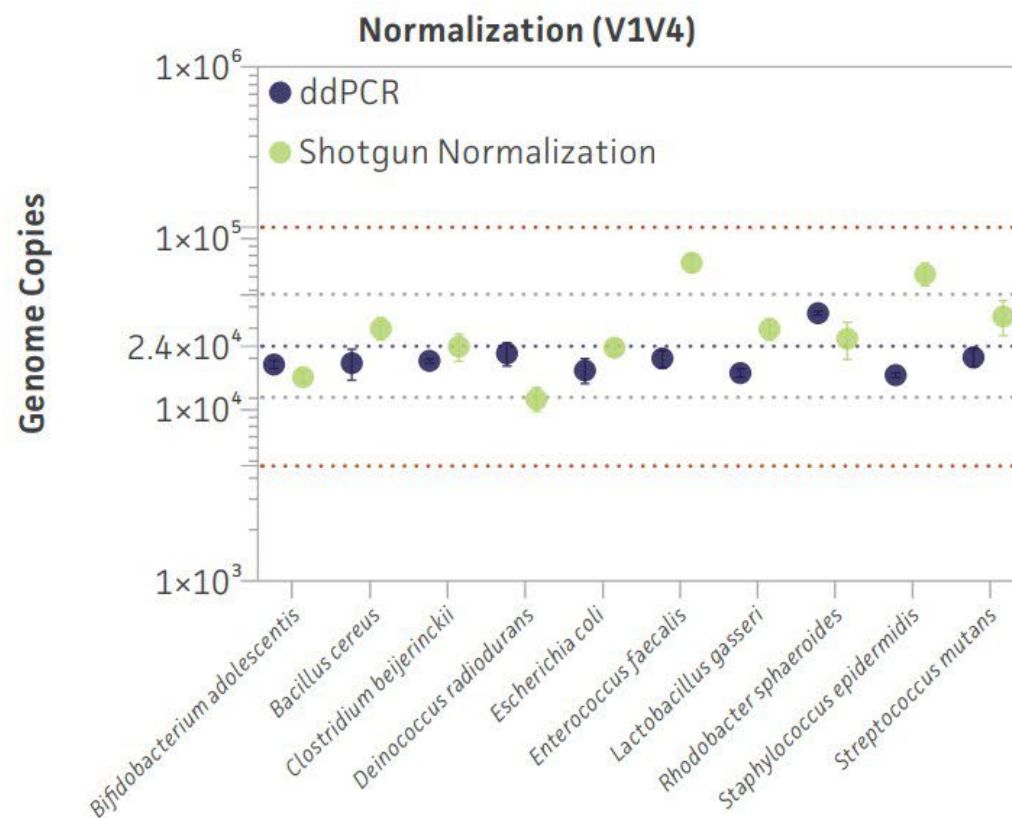
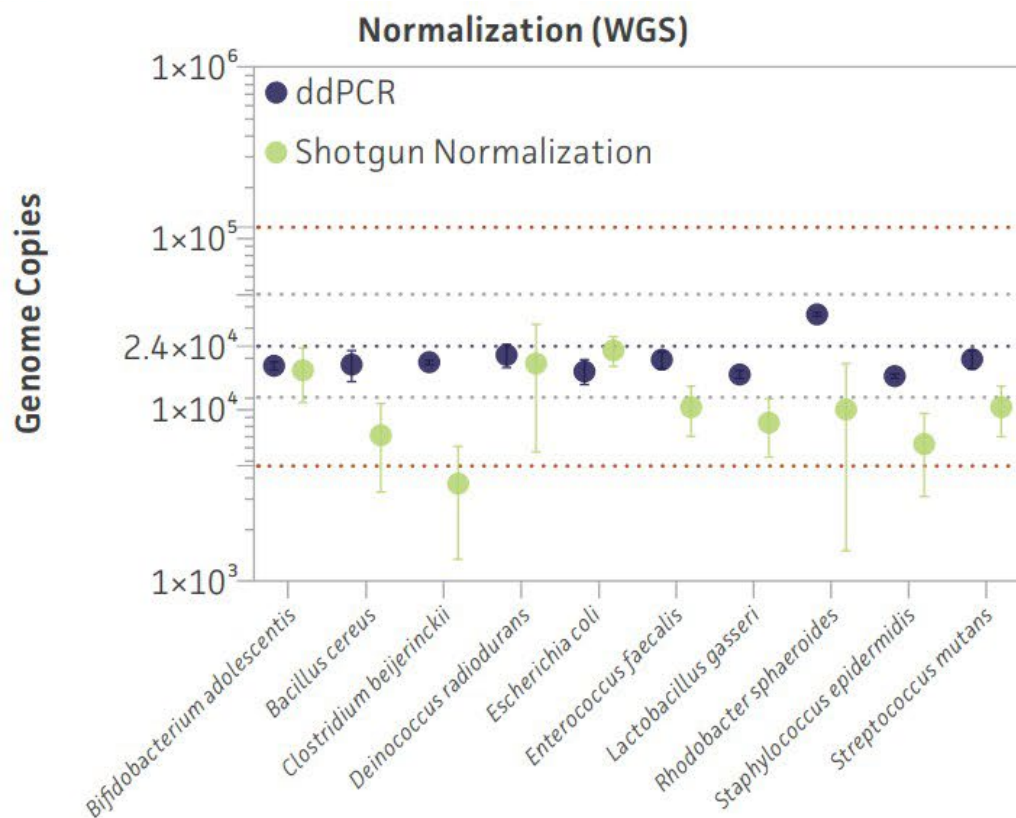
The spike-in doesn't have an obvious impact on whole genome shotgun analysis



ATCC[®] MSA-1014[™] was mixed with MSA-1000[™] at ~ 1:10 and ~1:100

Comparison of Absolute Quantitation by ddPCR and Normalized WGS

Three tagged genomic DNA mixed with 10 even genomic DNA (ATCC[®] MSA-1000[™])



ATCC Data Analysis Solution



Mock Microbial Communities

- Genomic DNA and whole cell standards
- Even and staggered mixtures comprising 10 or 20 strains
- Environmental and pathogen mixtures



Site-specific Standards

- Genomic DNA and whole cell standards
- Even mixtures of 6-12 strains
- Bacterial strains prevalent in the oral, skin, gut, and vaginal microbiome



Spike-In Standards

- Recombinant strains with a unique DNA tag stably integrated into the chromosome
- Recombinant standards include the Gram-negative and Gram-positive bacteria



Virome and Mycobiome Standards

- Genomic DNA and whole cell standards comprising diverse and clinically relevant strains
- Even mixtures of 6-10 strains

Bundled with data analysis on the One Codex platform



1. UPLOAD YOUR
DATA



2. SELECT YOUR NGS
STANDARD



3. ENTER YOUR
METADATA



4. REVIEW YOUR
RESULTS

ATCC NGS Standards for Microbiome Research

www.atcc.org/Microbiome

Microbiome Research

Raising the standards for
microbiome research

Optimize your research with the right controls

The complexities involved in 16S rRNA community profiling and shotgun metagenomics methods pose significant challenges for microbiome research. Significant biases can be introduced at each stage of the microbiome workflow, affecting data interpretation and reproducibility.

[NGS Standards](#) provide a solution to this problem. From sample collection to data analysis, NGS Standards enable you to optimize your diverse research applications with confidence and improve the consistency and reproducibility of your data, run after run.

The robust applicability of these controls, combined with the ATCC commitment to authentication and characterization, make NGS Standards ideal tools for standardizing data from a wide range of sources and generating consensus among microbiome applications and analyses.



Acknowledgements

- Joseph Leonelli
- Dev Mittar
- Briana Benton
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- Scott Tighe, UVM
- Pat Gillevet, PhD, Microbiome Analysis Center, GMU
- Rohan Patil, Microbiome Analysis Center, GMU
- Joan Wong, PACBIO®
- Tash Rodrigues, Diversigen
- Emily Hollister, Diversigen



Questions?

Credible Leads to Incredible™

